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FOOD RELATIONS OF WHEAT STEM SAWFLY (CEPHUS CINCTUS)

Neil Devlin Holmes

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PREFACE

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A PRELIMINARY STUDY OF FOOD RELATIONS OF WHEAT STEM SAWFLY (CEPHUS CINCTUS NORTON) LARVAE

INTRODUCTION

The wheat stem sawfly (Cephus cinctus Norton) is a major pest of wheat in Western Canada. In 1895 the first record of this species in Canada was made at Indian Head, Saskatchewan, by Fletcher (33). Since that time the area of infestation has spread until it now covers the brown and dark brown soil zones of Alberta and Saskatchewan.

A co-operative project between the Dominion Experimental Station, Swift Current, and the Dominion Entomological Laboratory, Lethbridge, was initiated to study sawfly resistance in wheat varieties. Kemp (65) found that the solid-stemmed varieties were more resistant to cutting than hollow-stemmed ones. A solid-stemmed, hard, red spring wheat, Rescue, was developed from a cross between two vulgare varieties, Apex and S-615. Rescue, which was grown on a field scale in 1946, showed a high degree of sawfly resistance in Alberta and Saskatchewan. However, at Regina, the resistance has not been satisfactory (80).

Platt et al (80) state that "variations in the nutrition of the plant, which in turn, might affect the nutrition of the insect" alone or in combination with other factors, may account for variations in the reaction of Rescue wheat to sawfly damage. Farstad[*] has noted that sex ratio, fecundity and form determination of the adult is influenced by the host plant (31). Because the nutrition of the wheat stem sawfly larva plays such an important role, Dr. Farstad believed that a study of this problem would provide a source of much valuable information. Therefore, at his suggestion, this project was started in the spring of 1947 with three main objectives:-

First, to study the tissues of the wheat plant which are ingested by the wheat stem sawfly larva,

Second, to determine the enzymes present in the gut of the larva, and

Third, to make a study of the morphology and histology of the organs of the larva which are engaged in the process of ingestion, digestion and excretion.

REVIEW OF INSECT NUTRITION

GENERAL LITERATURE REVIEW

The aim of this review is to provide a general back-

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[*] Dr. C. W. Farstad - Officer in Charge - Dominion Entomological Laboratory, Lethbridge, Alberta.

ground for a study of the nutrition of C. cinctus and not a complete outline of all the work on insect nutrition. It has been arranged under eight headings. The first deals with enzymes produced by insects, the following five sections outline the nutrients utilized by insects and the final two deal with the effect of nutrients on fecundity, sex and form determination.

ENZYMES

Enzymes are organic catalysts produced as a result of cellular activity, but are independent of the presence of living cells in their operations. They show the properties of colloidal systems. Hydrolytic enzymes that have been crystallized are protein in nature, while oxidizing enzymes are usually made up of a protein carrier of high molecular weight joined to a simpler group, the prosthetic group (53). In general the digestive enzymes of insects resemble those of other animals (107).

Organic nutrients must be directly assimilable by the tissues of the insect (glucose or natural amino acids) or they must be hydrolyzed to assimilable components by the digestive system in order to provide the requirements for energy, growth and reproduction (107). The ability to hydrolyze the organic materials lies in the enzymes. The enzyme system of an insect is a limiting factor to the range of diet upon which it can exist.

Hydrolytic enzymes are produced mainly in the cells of the salivary glands, the mid-gut and the various diverticula or caeca that are attached to it (107). Enzymes may also be produced by symbiotic organisms that may be found in the gut or in accessory structures (16, 17, 96, 103).

The salivary glands most commonly produce amylase and invertase only, while the mid-gut commonly produces the majority of the enzymes required for digestion. Gastric caeca or diverticula from the mid-gut increase the secretive area in many insects. Changes in the production of enzymes may be evident during the development of the insect. For example, caterpillars produce a wide range of enzymes, while the adults (Lepidoptera) may produce invertase only (107).

The cells producing digestive enzymes may act in unison, (synchronous), and a single cell may have a single, (monophasic), or several, (polyphasic), periods of activity. The production of enzymes in carnivorous insects is usually monophasic and synchronous, while in herbivorous insects, usually the enzyme production is polyphasic and asynchronous (18).

Insects as a class possess an extremely wide range of digestive enzymes, although an individual insect may have a single enzyme only (e.g. adult Lepidoptera). The following is a partial list of the hydrolytic enzymes that have been reported.

A. Esterases

1. CHLOROPHYLLASE: The chlorophyll of plants is partially broken down in the gut of the silkworm and other caterpillars (107) as well as in the intestine of *Forficula auricularia* (103).

2. LIPASE: is present in most necrophorous and carnivorous insects (107), as well as in *Periplaneta* and others (18, 103).

3. WAX-DIGESTING ENZYME: is reported to be produced in the intestine of the wax moth larva, *Galleria mellonella* (18, 103).

B. Proteases and Peptidases

1. RENNIN: is found in *G. mellonella*, *Coccinella* and *Cantharidae* (103).

2. PROTEASE: has been found in a large number of insects (9, 76, 84, 103). Parkin (76) reported protease to be present in fourteen species of beetles from the following families:- *Scolytidae*, *Cerambycidae*, *Lyctidae*, *Bostrychidae* and *Anobiidae*.

3. TRYPSIN: occurs in many insects (18, 103), among which are the cockroach *Blatta orientalis* (99), the cattle grub *Hypoderma lineatum* (94) and larvae of the Lyctid, *Hylesinius fraxinus*(61).

4. EREPSIN: is reported to be produced by *B. orientalis*, *Calliphora* and *Lucilia* larvae (99, 103) and larvae of the oriental

fruit moth, *Grapholitha molesta* (100).

5. 'KERATINASE': acts on keratin which is the chief constituent of hair and feathers. The S-S linkages are broken by a strong reducing agent and the resultant peptides are hydrolyzed by proteinase (107). Clothes moths, *Dermestes* larvae and species of *Mallophaga* produce a strong reducing agent capable of breaking the S-S bonds (18, 43, 103).

6. CHITINASE: is present in the Tenebrionid, *Platydema tricuspis* and in the larva of *Pseudagenia carbonaria* (Psammocharidae) (111).

(7. PEPSIN: does not occur in insects (107))

C. *Carbohydrases*

1. CELLULASE: which is limited to *Protozoa*, *Mollusca*, and *Arthropoda*, has been reported associated with ~~from~~ termites (16), *Coccidae*, phytophagous *Orthoptera* (111), eleven species of wood-boring beetles (76), and from a beetle, *Chlorophorus annularis*, which infests bamboo (73).

2. AMYLASE: is a very common enzyme, being produced by insects (73, 75, 84, 103).

3. INVERTASE: has been reported from species of the *Lyctus* powder post beetles (75) and *H. lineatum* (94) as well as others.

4. MALTASE: is present in the guts of *B. orientalis* (99) and *H. lineatum* (94).

5. LACTASE: has been demonstrated to be present in thirteen species of wood-boring beetles (76).

6. HEMICELLULASE A: is secreted by twelve species of wood-boring beetles (76).

7. HEMICELLULASE B: is produced in thirteen species of wood-boring beetles (76).

Fraenkel (34) found that eight carbohydrates were hydrolysed by the digestive system of adult blowflies, although there were only two carbohydrates, sucrose and maltose, found in their normal diet. He explained the large number of carbohydrates hydrolyzed by the blowfly enzymes in the following manner. All disaccharides, trisaccharides, and glycosides are hydrolyzed by glycosidases whose action is determined by the identity of the sugar, the potential aldehyde carbon atom of which enters the glycoside link, and by whether combination occurs through the alpha or beta position. This means that five enzymes are capable of hydrolyzing all known disaccharides, trisaccharides and more complex glycosides, based on glucose, galactose and fructose. They would be alpha glucosidase, beta glucosidase, alpha galactosidase, beta galactosidase, and beta-h-fructosidase. This theory of enzyme action was first postulated by Weidenhagen (106).

In termites the cellulase is produced by protozoa living in the gut (17). Also, protozoa in the gut of the wood-boring roach, Cryptocercus punctulatus, produce cellulase and cellobiase

necessary for the nutrition of the host (96, 111).

CARBOHYDRATES

Insects differ widely in their carbohydrate requirements. The diet of some adult insects such as *Lepidoptera* (66) is exclusively carbohydrate, while adult *T. confusum* do not require carbohydrates (38). The larvae of the clothes moth, *Tineola bisselliella*, do not require carbohydrates (43), whereas larvae of the moths, *Ephestia sericarium*, *E. elutella*, *E. cautella*, and *Plodia interpunctella* all require carbohydrates in their diet (44). Beetles of the genera *Stegobium* and *Lasioderma* require carbohydrates, while those of the genus *Ptinus* do not, and are thus able to infest a much wider range of food materials such as tobacco, yeast, museum specimens, drugs, and wool fabrics (38).

Carbohydrates are an important factor in the longevity of flies (103), and play a large part in the diet of the larvae of the European corn borer, *Pyrausta nubilalis* (8, 9). *Tenebrio molitor* larvae do not grow at all on a carbohydrate-free diet (102). At least some insects are capable of utilizing carbohydrates to synthesize fat, starved larvae of *Aedes aegypti* form glycogen and fat reserves when fed on carbohydrates alone (108).

Hive-bee larvae can live a relatively long time on a pure carbohydrate diet, although carbohydrates alone are not sufficient for growth (55, 70). The order of efficiency with which

hive-bee larvae could utilize carbohydrates ~~is~~ was as follows:- sucrose; maltose, melizitose, dextrose; trehalose, dextrins; galactose, lactose; starch and glycogen (5).

The southern armyworm, *Prodenia eridania*, when fed on young bean leaves utilized 56 per cent of the reducing sugars, 99 per cent of the sucrose and none of the starch (21). The larva of the mealworm *T. molitor*, when fed on a bran diet, was able to assimilate 46 per cent of the fructose, 67 per cent of the glucose, 59 per cent of the starch and 36 per cent of the hemicellulose (28).

Caterpillars of the species *Malacosoma disstria* utilized starch while *Aglais urticae* and *Pieris brassicae* were unable to do so. The caterpillars of all three species utilized a higher proportion of the sucrose in their food than of glucose, and none of them utilized cellulose or hemicellulose (26).

Brown (11) found that the grasshopper, *Melanoplus bivittatus* used monosaccharides completely but polysaccharides passed through the gut practically unchanged. He found that this grasshopper was unable to utilize starch grains.

Cleveland demonstrated that termites were capable of living longer than eighteen months on a diet of pure cellulose (16, 17).

PROTEINS AND AMINO ACIDS

Little is known of the amino-acid requirement of insects,

although it is known that insects, like vertebrates, require proteins or other nitrogenous foods for growth and reproduction. Many adult insects which have adequate reserves can maintain life for considerable periods in the absence of nitrogenous foods. However the growing stages and some adults without adequate reserves require proteins or their equivalent (102, 103).

Parkin (76) found that all the fourteen species of beetles which he examined from the families Scolytidae, Cerambycidae, Lyctidae, Bostrychidae and Anobiidae, digested protein. From this evidence he postulated that the larvae of all wood-boring beetles required protein in their diet.

Proteins as well as carbohydrates are required by the adult hive-bee (70) and by the larvae of Tenebrio (102). Keratin, the chief constituent of hair and feathers, forms an important part in the diet of the clothes-moth larvae, Dermestid beetles and feather lice (43, 107, 111).

Larvae of the blow fly, Calliphora erythrocephala, show a high degree of specificity for the proteins of higher vertebrates especially mammals. When fed on a diet of proteins from the flesh of birds their growth was slowed down, while a diet of proteins from the flesh of amphibia and fish resulted in retarded growth and high mortality. Pure proteins, such as egg albumen, peptones and amino acids alone were not sufficient for normal growth (67).

Larvae of Lucilia sericata require the addition of 3 mgms. of cystine per ml. of sterile medium to produce normal

puparia and complete development (71).

Bernard and Lemonde (4) found that the ten amino acids required by mammals could not replace casein in an artificial diet suitable for the growth of *T. confusum*, *T. molitor* and *Trogoderma ornatum*.

The larvae of *Drosophila melanogaster* grow as well on nucleotides as on ribonucleic acid. The growth promoting effect comes from the purines and pyrimidines especially in adenine, but not from ribonucleic acid as such. Guanine appears to have some effect in regulating the growth action of adenine. Desoxyribonucleic acid inhibits the growth and pupation of *D. melanogaster* (104)

D. melanogaster larvae require casein hydrolysate or an equivalent mixture of amino acids (laevo forms) to support normal growth. Ribose nucleic acid is important and can be partially replaced by adenylic acid, but not by the purine or pyrimidine bases (90).

Only the simplest amino acids are essential for the *B. germanica*. The nymphs are capable of living for six months on a pure carbohydrate diet, but will not grow unless amino acids are also provided (103). On the other hand amino acids were found to have no effect on the growth of larvae of the mosquito *A. aegypti* (52).

As termites can live for at least eighteen months on a pure cellulose diet, and carry out normal growth and reproduction,

it is believed that atmospheric nitrogen is fixed by the insect (16).

When starved larvae of A. aegypti were fed on a diet of casein, they replenished their reserves of fat, glycogen and protein (108). Thus protein may act as a source of material from which the insect may synthesize its carbohydrate and fat requirements.

LIPIDS

Lipids include not only the true fats, but substances that are chemically related to fats, or related to them because of common solubilities and possible biological relationships, such as lecithin and cholesterol (53).

Fatty substances are used for energy, stored in the fat body, and do not take a critical part in tissue building. Fats may be synthesized from proteins or from carbohydrates (103).

Lipase, the enzyme which hydrolyses lipids, is present in most carnivorous and necrophorous insects (107). Lipase is produced by the olive fruit fly Dacus oleae (103), the cattle grub Hypoderma lineatum (94), and many other insects (18).

The best food medium for P. nubilalis is one which contains fat (9), whereas the larvae of T. bisselliella do not require fat in their diet (43). Larvae of the beetle T. molitor utilize 83 per cent of the fatty acids present in bran (28) and wax moth larvae utilize approximately 50 per cent of the beeswax in their diet (107).

Larvae of the stored product pests *E. sericarium*, *E. elutella*, *E. cautella* and *P. interpunctella* all require linoleic acid in their diets in order to produce normal scales and to ensure normal emergence of the adults. Linoleic and linolenic acids have an equal effect, whereas oleic acid has no effect (46). Cholesterol is essential for all four species (44).

Arachidonic acid, unlike linoleic acid, has no positive effect on the development of the wings of *E.* SERICARIUM *sericata*, but has a positive effect on growth. In both respects it resembles the effect of docosahexaenoic acid from cod-liver oil. Linoleic acid is synthesized by *T. molitor* but not by *E. serica~~ta~~*Rium (47).

Cholesterol or another adequate sterol has been demonstrated to be essential in the diets of *T. confusum* (38), *Attagenus piceus* (56), *Dermestes vulpinus* (35, 50), *Silvanus*, *Stegobium*, *Lasioderma* and *Ptinus* (38). Cholesterol may be replaced in the diet by several related sterols. *Tribolium*, which requires no other fat soluble substance than a sterol, can utilize ergosterol equally well as cholesterol, but cannot utilize vitamin D (40). *D. vulpinus* larvae can utilize the animal sterols, cholesterol, 7 - dehydrocholesterol, cholesteryl acetate, and 7 - dehydro - cholesteryl monobenzoate for growth. However they cannot utilize plant sterols and are thus limited to foods of animal origin (35).

Fat may be synthesized by the cockroach, *B. germanica*, as the iodine number of the body fat is lower than that of the

fat ingested in the diet (18). The beet webworm, Loxostege sticticalis, is not capable of synthesizing linoleic acid, but must obtain it from the diet (77).

When starved larvae of the clothes moth are fed a carbohydrate or protein diet, their fat reserves are replenished. When fed a diet of fat, their fat reserves were replenished but the fat was not used to synthesize proteins or carbohydrates (108).

MINERALS

It is possible that mineral salts may constitute a limiting factor in the growth of insects on some diets (107). Various salts are essential for most insects.

Calcium is especially striking in its effect except with D. melanogaster larvae which appear capable of developing without it (102). Calcium has a favourable effect on the survival of the mosquito, Anopheles, for the first three days of its larval life (3). Larvae of the mosquito Culiseta incidens require calcium for growth and to a lesser extent magnesium sulfate, sodium chloride, or potassium iodide (49). Larvae of the mosquito, Aedes concolor, require calcium for normal development and calcium chloride is necessary for the normal growth of A. aegypti (102).

Sodium is required in the diet for the normal growth of the larvae of the mosquito A. concolor but sodium chloride has no effect on the growth of A. aegypti larvae. Sodium is not required

by Drosophila larvae for muscular work (102).

A. aegypti larvae do not require potassium chloride for normal growth, whereas Drosophila larvae require potassium in their diet for normal development but not for muscular activity (102).

Vegetarian insects have a high potassium concentration and carnivorous insects a high sodium concentration in their coelomic fluids. The ratio of sodium to potassium is a species characteristic and is normally governed by mechanisms of mineral regulation and not by the diet (7).

The rate of development of Lucilia larvae is proportional to the percentage of phosphorus in the diet (102). Similarly the time of pupation for Tribolium is inversely proportional to the amount of phosphorus in the diet. The percentage of phosphorus in the pupa of T. confusum is constant despite the percentage of phosphorus in the diet (72). Drosophila larvae require phosphoric acid for development (102).

Haseman (54) reported that the chinch bug, Blissus leucopterus, and greenhouse thrips, thrive better on plants which are deficient in nitrogen.

Dahms (22) found that B. leucopterus laid more eggs on Atlas sorgo and Finney milo seedlings growing in a nutrient solution containing a high percentage of nitrogen. When these seedlings

were grown in a solution in which the nitrogen-phosphorus ratio was low the average egg deposition was also low. A high nitrogen content of spinach is positively correlated with lowered attack by thrips, but an increase of calcium in spinach which has a low nitrogen content reduces the effect of thrip attack (110).

Grain aphids were only capable of surviving for a few generations on wheat which was deficient in nitrogen (54). In twenty-four hours the termite, Kalotermes flavicolis, is capable of synthesizing nitrogen in amounts equal to 65 per cent of its own weight (101).

The caterpillars Alabama argillacea require copper and zinc in their diet (19), and the rat flea Nosopsyllus fasciatus needs iron for normal growth (93).

ACCESSORY FACTORS

Unless all the complex organic substances in the body are synthesized by the insect from raw materials, these substances themselves, or special precursors for them, must be provided in the diet. These are called accessory factors (107). Most insects studied so far require only one fat soluble factor which is a sterol. However the sterols which are required, cholesterol, ergosterol, or related sterols, cannot be replaced by the sterols of the vitamin D group. Many of the water-soluble vitamins are required by both insects and vertebrates (102). Vitamins may be completely or partially supplied by symbiotic micro-organisms in the gut of the insect (96, 102, 107).

Vitamin A may be required in the nutrition of the European corn borer (9) and the cockroach, *B. germanica*, can thrive on a diet containing vitamin A (10). On the other hand, the confused flour beetle can live on a vitamin A-free diet (72).

Vitamins belonging to the B complex have been demonstrated to be important accessory factors in the nutrition of insects. The addition of total B complex to white flour in the larval diet of the confused flour beetle increases the rate of larval development and pupal weight. When added to the diet of starved adults of *T. confusum*, their length of life was increased (89). Blowfly, *Calliphoridae*, larvae resemble higher animals in their B complex requirements. The chief effect of a deficiency of B complex from the diet is retarded larval growth. Blowflies are incapable of developing aseptically on a sterile diet without vitamins of B complex added (60).

Thiamine chloride is essential in the diet of the confused flour beetle (37, 97, 98) and in the diet of the larval stage of the mosquito *A. aegypti* (13). Larvae of the beetles *Lasioderma serricorne* and *Stegobium paniceum* require thiamine in their diets (40). Royal jelly, which is fed to queen bees, exhibits vitamin B^1 activity equal to approximately 9 micrograms per gram of dry matter. Bee bread exhibits B^1 activity equal to 6.4 micrograms per gram dry matter (57). (One unit of B^1 potency is exerted by 3 micrograms of crystalline thiamine chloride). When larvae of *T. confusum* are fed a thiamine-deficient diet the

fat body consists of small cells poor in fat. Rice moth larvae, *C. cephalonica*, when fed on a diet deficient in thiamine, develop degenerative changes in the cells of the fat body, and accumulate large amounts of pyruvic acid (88) which disappear on the addition of thiamine chloride (86, 64).

Riboflavin is an essential growth factor in the diet of *T. confusum* (2, 83), *L. serricorne*, *S. paniceum*, *E. sericarium* (37, 44) and *A. aegypti* (24). The rate of growth of the rice moth larva is proportional to the amount of riboflavin in the diet up to 1.2 micrograms per gram of diet (87).

Niacin is a necessary factor in the diet of the clothes moth larva (43) of the stored products pests *E. sericarium* and *E. elutella* (44). When niacin is added to the diet of *T. confusum*, the larval period is shortened by one-third (2), and when added to the diet of *A. aegypti* growth to the fourth instar is ensured (24). Symbionts of the beetles *L. serricorne* and *S. paniceum* supply their hosts with niacin (39). Sarma states that apparently niacin is not required in the diet of the larval stage of the rice moth (87) although Rubinstein and Shekun (85) report that it is necessary.

Folic acid, another of the B complex vitamins, and which is synthesized by micro-organisms in the human gut, is also synthesized by intestinal bacteria of the bed-bug, *Cimex lectularis*, (25) and may be synthesized by bacteria in the gut of *T. confusum* (109). Folic acid is essential to larvae of *A. aegypti* in order to ensure pupation (24) and must be present in the diet not later

than the third instar (52). Two micrograms of folic acid per gram of dry weight of diet is required by *E. sericarium*, and is necessary in undetermined amounts in the diets of the beetles *Tenebrio molitor* and *T. confusum* (45). The rate of growth is very slow if folic acid is absent from the diet of *E. sericarium*, although xanthopterin has the same effect as folic acid if supplied in quantities one thousand times greater than those of folic acid (48).

Pyridoxine is essential in the diet of *T. confusum* in order to ensure pupation (2, 83). The larvae of the rice moth (87), of *E. sericarium* and of *E. elutella* all require pyridoxine (44). The absence of pyridoxine results in retarded growth of the clothes moth (43). The symbionts of the larvae of the beetles *L. serricorne* and *S. paniceum* normally supply pyridoxine to their hosts (39).

Pantothenic acid is another of the B complex which is required by *T. confusum* (82), *T. bisselliella* (43), *E. sericarium* and *E. elutella* (44), in order to ensure growth. Pantothenic acid is not required by larvae of the rice moth (87) but it is necessary to ensure growth of *A. aegypti* to the fourth instar (24). The symbionts of the larvae of *S. paniceum* and *L. serricorne* normally supply pantothenic acid to their hosts (39).

Biotin has been demonstrated to be an important growth factor in the diets of the moths *E. sericarium* and *P. interpunctella*

and is less essential to *E. elutella* (44). It is of doubtful value for larvae of *T. bisselliella* (43) but may be essential to ensure growth to the fourth instar of the mosquito *A. aegypti* (24). The optimum requirement of *T. confusum* larvae for biotin is 0.05-0.1 micrograms per gram of dry diet (41). Pimelic acid and desthiobiotin may replace biotin for *T. confusum*, but are required in concentrations twice as great as that required of biotin (81).

Inositol has some effect in increasing the growth rate of *E. sericarium* (44), but the clothes moth can develop without it (43).

Para-amino benzoic acid is not required by *T. confusum* (82), *T. bisselliella* (43), *E. sericarium* and *E. elutella* (44).

Ascorbic acid, vitamin C, has no effect on the larva of A. aegypti (24), but has a positive effect on the growth of *Ephestia* species. Ascorbic acid acts as an anti-oxidant on the saponifiable fraction of wheat germ oil (46).

Vitamin D is not required by *T. confusum* (15, 72), nor by the larvae of the beetle *D. vulpinus* (35).

Vitamin E, alpha tocopherol, added to the diet has no effect on the growth of *T. confusum* and *P. interpunctella* (40, 42). Vitamin E acts as an anti-oxidant in stabilizing the linoleic acid, in wheat germ oil, which is required by the moths:- *E. sericarium*, *E. elutella*, *E. cautella* and *P. interpunctella*, in order to ensure good emergence and good scales on the wings. Vitamin E also has

a growth effect upon these moths (46). Hill and Burdett (59) claimed that vitamin E was present in royal jelly which is fed to queen bees; but this claim was disproved in later work by Evans et al (29). However it has been shown that royal jelly does contain a higher percentage of pantothenic acid than the food fed to worker larvae (102).

The intracellular symbionts of L. serricorne and S. paniceum provide their hosts with thiamine chloride, riboflavin, niacin, pyridoxin and pantothenic acid (6).

THE EFFECT OF NUTRITION ON REPRODUCTION

In most holometabolous insects all or most of the nutrients required for reproduction are accumulated during larval life. These quantitative and/or qualitative differences in larval feeding affect both the duration and fecundity of the resultant adults. When the reproductive organs of the adult are not fully developed on emergence, both the feeding of the adult as well as larval nutrition affect its reproductive ability (102).

The fecundity of females of Cecidomiid species varies inversely with the number of larvae per floret and is also affected by the food plant and conditions of moisture under which the plant is grown (74).

Inclusion of yeast in the diet of Tribolium increases the egg production of the females (68).

The pH of cell sap of eleven varieties of trees had an effect on the fecundity of aphids, acidity decreased and alkalinity

increased fecundity (27, 63).

Under late summar conditions of light the rate of reproduction of the aphid Brevicoryne brassicae is positively correlated with the protein content of the plant on which it feeds (27). A single wingless aphid placed on a young bean plant with the growing point intact produced 335 offspring, while a single wingless aphid on a bean plant with the growing point removed produced in the same period 105 offspring (23).

Haseman (54) showed that grain aphids, Macrosiphum granarium, produced few offspring where there was a deficiency of iron or nitrogen, and produced more offspring where deficiencies of sulphur, potassium, magnesium or phosphorus occurred.

THE EFFECT OF NUTRITION ON FORM DETERMINATION

The quantity and quality of food available during the growing stages of an insect may affect the size, sex and various other characters such as eye colour and wing proportion (102).

Holdaway (62) working with T. confusum larvae advances the following alternate theories concerning the effect of nutrition on sex.

(a) A proportion of the population is stable with regard to sex, while a proportion is more easily converted to the opposite sex.

(b) Forms of either sex may be changed to the opposite sex, but there are gradations of sexual stability that are upset

only after varying periods of time.

The effect of nutrition on sex determination takes place through its action on the biochemical and biophysical state of body fluids and also on germ cells. There are at least gradations in constitution which show a relation between nutritional status dependent on time of starvation and ultimate sex determination.

Vitamins may play an important part in sex and form determination. Quantitative larval feeding of the fly, *Lucilia sericata*, influenced the sex of the adults. The sex ratios when fed on different amounts of yeast were as follows:- one gram of yeast gave 3.3 males to 6.7 females, while four grams of yeast gave 5.5 males to 4.5 females (58). Goetsch (51) showed that vitamin 'T complex' would induce the formation of soldier castes in ants and termites which previously had none, and also induce gigantic- or big-headed forms in certain other insects, often with an increased need for oxygen and with increased pigmentation.

Protein in the larval diet may be a factor. The formation of wingless forms of the aphid *Brevicoryne brassicae* is negatively correlated with the protein content of the plant host (27). Aphids feeding on a young bean plant with the growing point intact produced a high proportion of winged individuals, while those which fed on a bean plant with the growing point removed produced a small proportion of winged individuals. (23).

REVIEW OF LITERATURE ON THE NUTRITION OF C. CINCTUS

A general review of the literature on insect nutrition indicates that the insects most commonly studied are those which feed on non-living materials such as flour and other stored food products. Although progress on the nutrition of external plant feeders is not great, even less work has been reported concerning the relation of nutrients to mining and boring insects. All the literature on *Cephus* deals with the differences between varieties of wheat in their influence on *C. cinctus* as shown by Farstad (31) and Farstad and Platt (79, 80). The reason for this paucity of literature on insects which confine their feeding inside a plant is mainly due to the difficulty of preparing artificial media in a form which is acceptable to the insect.

Farstad (31), Platt and Farstad (79, 80) have shown that varieties of wheat influence the size, fecundity, and sex ratio of surviving populations. The succulence (moisture content) of the host plant influences the length of the larval feeding period. The size of the larva is increased with an increase in the period of feeding, and the size of the larva is positively correlated with fecundity and size of the adult. The number of instars is influenced by the host plant.

The sex ratio of *C. cinctus* is influenced by the variety of the host (31). In the Nobleford area no males had been found for several years although both sexes commonly occur in other areas (30).

Farstad (31) found that newly hatched *C. cinctus* larvae will die unless they can start feeding on the succulent tissues near the area of active elongation. Larvae from eggs laid near the node will survive, while those from eggs laid near the head, in the same internode, will die. The female *C. cinctus* will oviposit readily in oats, although this plant shows a ~~complete~~ lack of complete nutritional requirements for growth and development of the larva. Farstad also reports that the larvae will die when the eggs from which they hatch are completely surrounded by pith. It appears that the pith itself lacks essential growth factors, and if firm, the larva may be forced to expend too much energy in obtaining a sufficient supply of nutrients to maintain life.

LIFE HISTORY

The life history and habits of the sawfly have been outlined by Seamans (91, 92) and Criddle (20). Adults emerge from early June until mid-July and immediately commence oviposition. The eggs are laid in the lumen of wheat stems that are preferably in the "boot" stage. The larva, reported by Farstad (31) to have five instars in Marquis wheat, feeds on the inside of the stem until the plant reaches maturity. It then migrates to the base of the culm and girdles it at ground level. The exposed end of the stub is plugged with frass and a hibernaculum is produced. The mature larva is fully mobile within the stub during hibernation.

During late May and early June it pupates and emerges as an adult.

The hibernaculum, which has been described as a "silken case", is a transparent light-brown sheath resembling "cellophane" in texture and appearance. The use of the word "silken" is inappropriate as it implies that the case is composed of fibres, while the construction is actually that of a continuous membrane. Tests with iodine and sulphuric acid gave negative results which indicate the hibernaculum is not composed of cellulose. During August 1948, newly cut stubs were examined and found to be without hibernacula. A short time later, stubs from the same fiele were again examined and hibernacula were found in all stubs. It is quite possible that they may be produced by the labial glands.

MORPHOLOGY AND HISTOLOGY OF THE SAWFLY LARVA

MATERIALS AND METHODS

Larvae were collected from wheat in the area of Lethbridge, Alberta. Fourth and fifth instar larvae, which are approximately one-half inch in length, were chosen as the most feasible size for purposes of dissection. Bouin's solution was used as the killing and preservative agent. Microtome sections were made from paraffin-imbedded material, and whole mounts were prepared from larvae cleared in oil of cloves. All nomenclature used is that of Snodgrass. (95).

THE LARVAL ORGANS OF INGESTION, DIGESTION AND EXCRETION

No previous work has been done on the internal morphology of any of the immature stages of *C. cinctus*. The feeding or immature stage of the larva has been selected for study, for upon reaching maturity, all feeding ceases, the gut is reduced to a thread-like organ, and fat body fills the perivisceral sinus.

ORGANS OF INGESTION

The mandibles are strong biting jaws of the ordinary phytophagous type possessing well defined incisor and molar regions (figs. 8, 9). Each is dicondylic with the outer edge of its triangular base strongly articulated at the pleurostomal region of the cranium. The musculature of the mandible consists of a dorsal abductor inserted on an apodeme attached to the outer margin of the mandible, and a dorsal adductor inserted on an apodeme attached at the inner angle of the mandible (fig. 9).

The maxillae are closely associated with the labium and hypopharynx to form an under-lip complex.(fig. 10). Each maxilla consists of a distinct cardo and stipes, the latter bearing on its ventral extremity the lacinia, galea, and palpus (fig. 12).

The labium is a simple structure consisting of a basal postmentum and a distal prementum, both of which are entirely membranous (fig. 11). The prementum bears, on the distal margin, the three-segmented palpi and the ligula. The latter is a single

flap bearing the orifice of the labial glands in its ventral surface.

The head capsule is strengthened along its subgenal region by a strong pleurostomal ridge bearing the mandibular articulations. Anterior to the mandibles, a pair of epistomal bars extend to the anterior tentorial pits. These bars are connected between the pits by the epistomal ridge which separates the clypeus from the frons (figs. 3, 4).

The tentorium consists of a pair of anterior arms which unite with the strong tentorial bridge, a broad structure originating in the post-occipital suture (figs. 5, 6, 7).

The labial glands (figs. 2, 24, 25) are a pair of elongate tubes which lie free in the body cavity at the sides of the alimentary canal. Each tube is U-shaped, the posterior ends terminating near the ventricular valve. The anterior part encircles the gastric caecum and is enlarged to form a reservoir. From the reservoir a duct leads forward to unite with the duct from the other reservoir, forming a common tube which has its outlet in the ventral surface of the ligula (fig. 25). The probable function of these glands is salivary as well as providing secretion for production of the hibernaculum. The labial glands, unlike the alimentary canal, are not reduced in the overwintering larva.

THE ALIMENTARY CANAL

The stomodaeum is little more than an inlet from mouth

to stomach (fig. 15). It consists of buccal cavity, pharynx, oesophagous, and proventriculus. The proventriculus is invaginated into the anterior end of the mesenteron to form the cardiac or stomodaeal valve. The stomodaeal epithelium is thrown into longitudinal folds, and is covered by chitinous intima (figs. 16, 17, 18). A sheath of circular muscles, external to which are longitudinal fibres, encircles the stomodaeum. Dilator muscles originate on the walls and apodemes of the head, the central ends inserted in the stomodaeal epithelium. The cardiac or stomodaeal valve which is composed of two cellular lamella covered on each side with stomodaeal intima, acts as a sphincter, preventing return movement of food.

The mesenteron, which is the middle section of the alimentary canal, functions as a stomach (figs. 2, 19). It consists of a large simple tube extending from the base of the outer fold of the stomodaeal valve at the termination of the stomodaeal intima, to the ventricular valve. Two gastric caeca which arise at the anterior end of the mesenteron are simple ovate processes connected to the mesenteron in the area surrounding the stomodaeal valve (fig. 18). The epithelial cells are columnar in shape possessing large nuclei, and a striated border which differentiates the central ends. The peritrophic membrane is continuous with the epithelium, and is formed from the entire surface of the latter. Enclosing the mesenteron is a sheath of circular fibres, external

to which are longitudinal muscles. At the posterior end of the mesenteron, the epithelium of the ventriculus projects into the lumen of the proctodaeum forming the ventricular valve (fig. 21).

The proctodaeum is differentiated into anterior intestine and rectum (fig. 22). The Malpighian tubules enter the proctodaeum immediately posterior to the ventricular valve (fig. 21). The walls of the proctodaeum consist of epithelium thrown into longitudinal folds covered internally with cuticular intima. In the rectum, six papilla-like organs project into the lumen. The proctodaeum is encased by a circular sheath of muscles overlaid with longitudinal fibres, and in the rectal area, dilator muscles originate in the body wall, their central ends entering the proctodaeal epithelium (fig. 23).

The larva possesses ten Malpighian tubules which empty into the alimentary canal through a central opening in the ventral surface of the proctodaeum (fig. 21). All the tubules lie free in the body cavity; four extend to the anterior end of the mesenteron, four caudally to the area of the rectum, and two extend in an anterior direction, reverse, and terminate in the caudal area (figs. 14, 20).

FAT BODY

The fat body in the perivisceral sinus is arranged in several long strands which extend almost the full length of the

body. These strands enlarge to fill the perivisceral sinus of the mature larva.

GONADS

Partially-developed gonads have been observed in the dorsal area of the fifth abdominal segment in mature larvae.

THE PYGIDIUM

Although not an organ of ingestion, the pygidium is used by the larva in obtaining food. It arises from the tenth or pygidial segment of the abdomen (fig. 1) and is a heavily sclerotized circular structure bearing teeth on its terminal surface (fig. 26). The pygidium acts as a brace to support the larva while it rips off tissues of the stem. This function will be discussed in a later section.

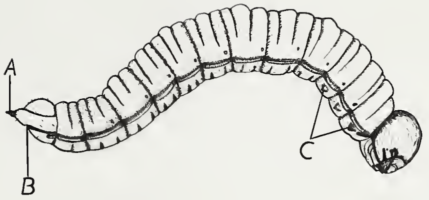

Fig. 1. - S-shaped larva. A, pygidium; B, anus; C, thoracic legs. (x 6)

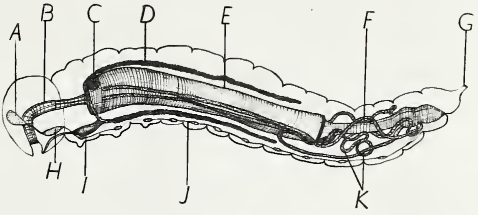

Fig. 2. - Diagram of the internal organs. A, brain; B, stomodaeum; C, gastric caecum; D, labial glands; E, mesenteron; F, proctodaeum; G, pygidium; H, suboesophageal ganglion; 1, reservoir of labial gland; J, ventral nerve cord; K. Malpighian tubules.

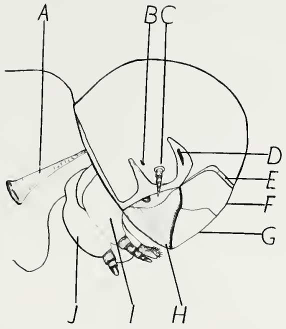

Fig. 3. - The head, lateral view. A, cervical sclerite; B, larval eye; C, antenna; D, anterior tentorial pit; E, epistomal suture; F, clypeus; G, labrum; H, mandible; I, maxilla; J, labium.

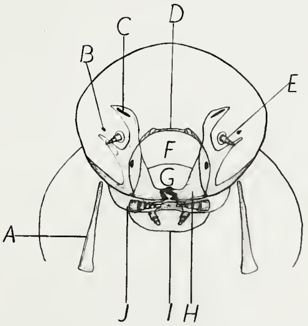

Fig. 4. - The head, ventral view. A, cervical sclerite; B, larval eye; C, anterior tentorial pit; D, epistomal suture; E, antenna; F, clypeus; G, labrum; H, mandible; I, labium; J, maxilla.

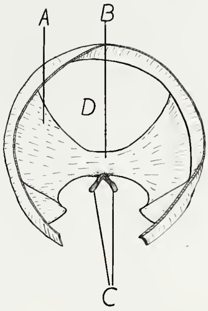

Fig. 5. - The tentorium, dorsal view. A, B, tentorial bridge; C, anterior tentorial arms; D, foramen magnum.

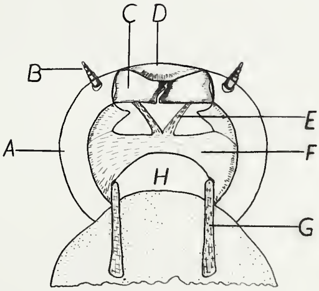

Fig. 6. - The tentorium, labium and maxillae removed, ventral view. A, gena; B, antenna; C, mandible; D, labrum; E, anterior tentorial arm; F, tentorial bridge; G, cervical sclerite; H, foramen magnum.

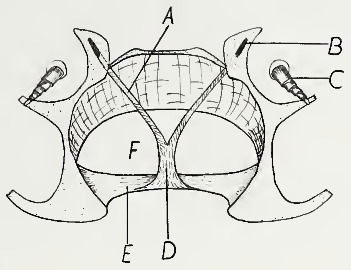

Fig. 7. - The tentorium, frontal view. A, anterior tentorial arms; B, anterior tentorial pit; C, antenna; D, corpora tentorium; E, tentorial bridge; F, foramen magnum.

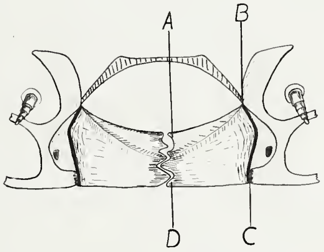

Fig. 8. - The mandibles. A, molar region; B, epicondyle; C, hypocondyle; D, incisor region.

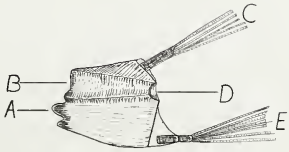

Fig. 9. - Right mandible. A, incisor region; B, molar region; C, dorsal adductor; D, epicondyle; E, dorsal abductor.

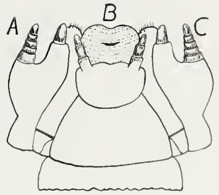

Fig. 10. - Maxillae and labium, ventral view. A, C, maxillae; B, labium.

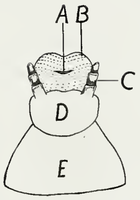

Fig. 11. - Ventral view of the labium. A, orifice of the labial glands; B, ligula; C, palpus; D, prementum; E, postmentum.

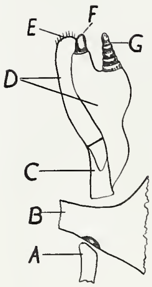

Fig. 12. - Maxilla extended, ventral view. A, cervical sclerite; B, tentorial bridge, C, cardo; D, stipes; E, lacinia; F, galea; G, palpus.

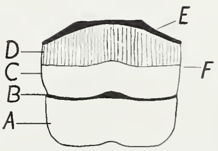

Fig. 13. - The clypeus and labrum, anterior view. A, labrum; B, clypeo-labral suture; C, ante-clypeus; D, post-clypeus; E, epistomal suture; F, clypeus.

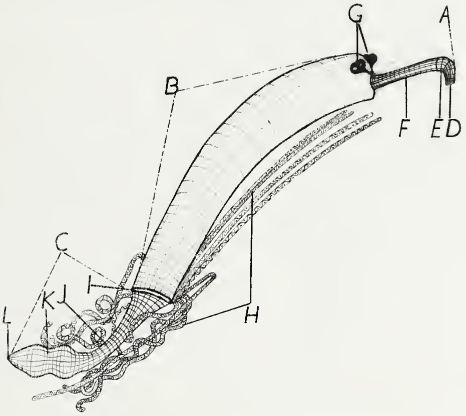

Fig. 14. - The alimentary canal. A, stomodaeum; B, mesenteron; C, proctodaeum; D, mouth; E, pharynx; F, oesophagus; G, gastric caeca; H, Malpighian tubules; I, ventricular valve; J, anterior intestine; K, rectum; L, anus.

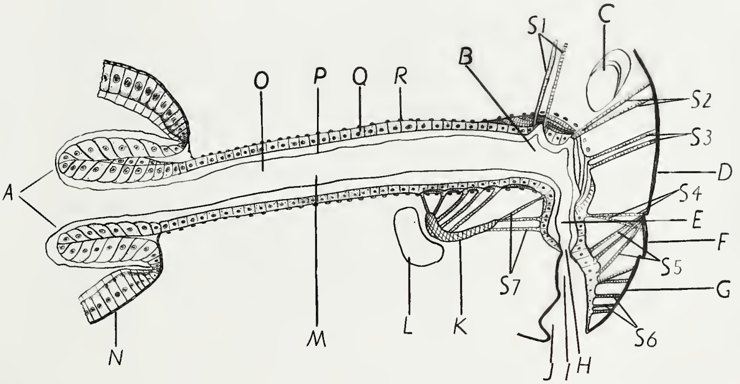

Fig. 15. - The stomodaeum, longitudinal section. A, stomodaeal valve; B, pharynx; C, brain; D, frons; E, buccal cavity; F, clypeus; G, labrum; H, mouth; I, cibarium; J, preoral cavity; K, tentorium; L, suboesophageal ganglion; M, oesophagus; N, mesenteron; O, lumen; P, intima; Q, epithelium; R, circular muscle; S.1, dilatores post-pharyngeales; S.2, dilatores pharyngis post frontales; S.3, dilators pharyngis frontales; S.4, dilatores buccales; S.5, dilatores cibarii; S.6, compressor labri; S.7, ventral dilatores.

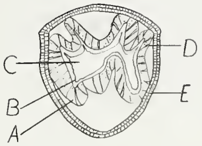

Fig. 16. - The pharynx, cross section. A, epithelium; B, intima; C, lumen; D, basement membrane; E, circular muscles.

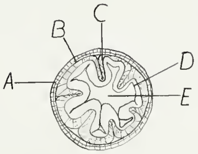

Fig. 17. - The oesophagus, cross section. A, circular muscles; B, basement membrane; C, epithelium; D, intima; E, lumen.

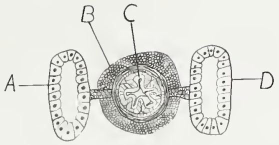

Fig. 18. - The gastric caeca, cross section. A, D, gastric caecum; B, mesenteron; C, stomodaeal valve.

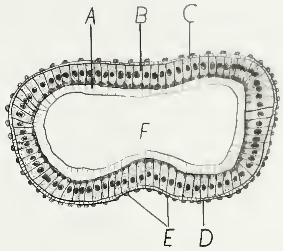

Fig. 19. - The mesenteron, cross section. A, peritrophic membrane; B, epithelium; C, basement membrane; D, circular muscle; E, longitudinal muscles; F, lumen.

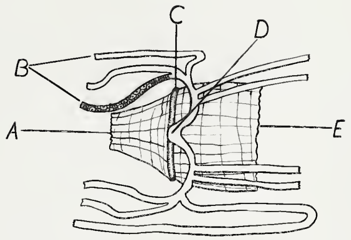

Fig. 20. - The Malpighian tubules, ventral view. A, proctodaeum; B, Malpighian tubules; C, ventricular valve; D, common entrance of the tubules to gut; E, mesenteron.

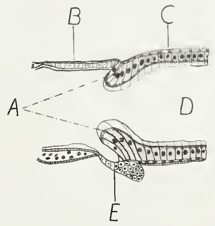

Fig. 21. - The ventricular valve, longitudinal section. A, ventricular valve; B, proctodaeum; C, mesenteron; D, lumen; E, entrance of Malpighian tubules to gut.

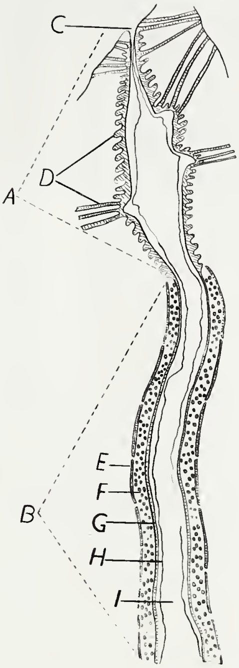

Fig. 22. - The proctodaeum, longitudinal section.
A, rectum; B, anterior intestine; C, anus;
D, dilator muscles;
E, longitudinal muscles;
F, circular muscles;
G, epithelium;
H, intima; I, lumen.

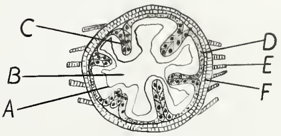

Fig. 23. - The proctodaeum (rectal area), cross section. A, intima; B, lumen; C, D, epithelium; E, dilator muscles; F, circular muscles.

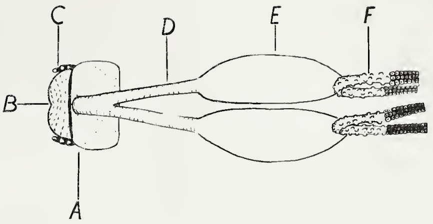

Fig. 24. - Diagram of the labial glands, dorsal view. A, labium; B, ligula; C, palpus; D, labial duct; E, reservoir; F, labial gland.

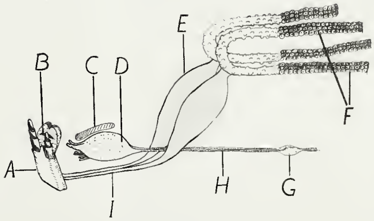

Fig. 25. - Diagram of the labial glands, lateral view. A, prementum; B, orifice of labial glands in the ligula; C, tentorium; D, suboesophageal ganglion; E, reservoir of labial gland; F, labial glands; G, prothoracic ganglion; H, ventral nervous cord; I, ducts of labial glands.

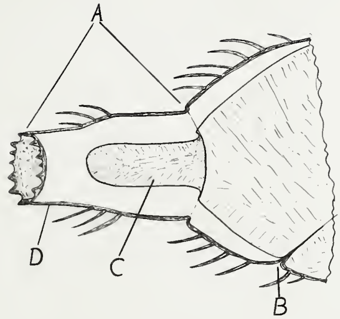

Fig. 26. - The pygidium, optical view. A, C, D, pygidium;
B, anus.

MORPHOLOGY OF THE WHEAT STEM

Since the larva obtains from the wheat stem all nutrients required for growth, development and reproduction, a knowledge of morphology of the culm is an integral part of the nutritional study of *C. cinctus*.

Percival (78) describes the anatomy of the wheat stem as follows:-

1. EPIDERMIS: made up of narrow elongated cells with short square cells at intervals.

2. HYPODERM: consists of lignified fibres with narrow lumina and strong walls.

3. ASSIMILATING PARENCHYMA: made up of delicate cells in the hypoderm and appear as parallel narrow green strips.

4. COLOURLESS GROUND PARENCHYMA: extends from the hypoderm to the hollow pith cavity. The cells which are thin-walled and finely-pitted, become thickened and lignified at the lowest internodes.

5. VASCULAR BUNDLES: are distributed symmetrically in the stem; very small ones located in the hypoderm, the large bundles in ground parenchyma. The large bundles are of the closed collateral type with xylem directed towards the centre of the stem, its chief vessels arranged to form a V. The protoxylem forming the apex of the V consists of one or two annular

or spiral vessels; two pitted vessels of metaxylem with wider lumina form the right and left arms of the V, and the space between the main vessels is occupied by small tracheids. The phloem made up of seive-tubes and companion cells, lies between the arms of the V. Sheaths of fibrous sterome surround each bundle. Generally at the node, all the small bundles in the hypoderm come from the leaf sheath immediately above, half the large bundles come from the leaf sheath, the other half descend through the node from the internode above. The total number of bundles is generally greatest in one of the two upper internodes, fewest in the middle of the stem.

The bundles of the leaf sheath curve inwards at the node and pass through the latter as separated strands that do not fuse with each other or with neighbouring bundles from the internode above. Around the bundle in its course through the node is a sheath of parenchyma containing many ~~green~~ chloroplastids. The xylem elements are increased within the node, the vessels being replaced by many short, pitted, and reticulately thickened tracheids of small diameter distributed irregularly with xylem parenchyma. In the lower section the xylem elements more or less completely encircle the bast, but this arrangement is lost after the bundles leave the node, and the collateral form is soon resumed (78).

The anatomy of the stem of the wheat variety Thatcher is very similar to that described by Percival (78), although green

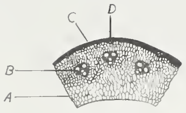

Fig. 27. - The wheat stem, cross section of internode. A, parenchyma; B, large vascular bundle; C, hypoderm; D, small vascular bundle. (after Percival, 78).

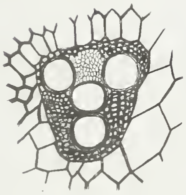

Fig. 28. - A large vascular bundle. (after Percival, 78).

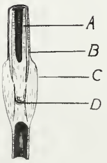

Fig. 29. - The node, longitudinal section. A, stem; B, leaf sheath; C, thickened leaf base; D, diaphragm. (After Percival, 78).

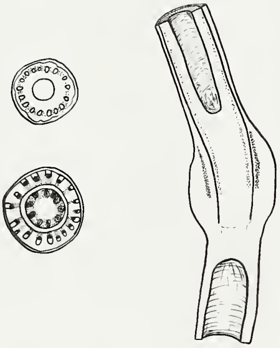

Fig. 30. - Starch grains in the stem. Longitudinal section through the stem, and cross sections of internode and node. (Thatcher variety) Dots indicate presence of starch.

assimilating parenchyma of the hypoderm disappears where it is covered by the leaf sheath (figs. 27, 28, 29). The node is solidly filled with pith containing green chloroplastids. These bodies are also found in the outer parenchyma of the internodes and in decreasing numbers in parenchyma cells towards the pith cavity. Starch granules were present in parenchyma between bundles at the node and part way up the internode (fig. 30).

FEEDING AND DIGESTION

The adult of *C. cinctus* emerges sexually mature, with a full complement of eggs. Thus the larval stage must obtain all nutrients required for growth, development, and reproduction. These nutrients are stored in the very large amounts of fat body possessed by mature larvae. The adult gut is reduced to a thread-like organ. Farstad (32) reports that adults have been observed feeding in flowers and are attracted by sugar solutions. However, in laboratory experiments, unfed females lay full complements of viable eggs.

SELECTION OF OVIPOSITION SITE

The female deposits the small crescent-shaped eggs in the lumen of the stem. Farstad (32) states that most eggs are deposited in the third internode, and females will oviposit more

readily in stems that are actively elongating (31).

Brome stems were split into east and west halves. Fifty stems were examined; 75 eggs were laid in the west section, 67 in the east. One hundred stems were split into north and south halves; 94 eggs were found in the north, 165 in the south half. Greater oviposition in the south half of the stem is probably related with sunlight, and perhaps with deposition of food materials in the stem.

FEEDING METHODS

Experiments on artificial feeding of C. cinctus larvae were initiated in 1948. Hollow glass tubing with a bore of 1.0 ~~0.5~~ mm. was ~~were~~ cut into 3-inch lengths. Sections of wheat stems, 3 inches long, were placed in the tubes, one half stem in each. Larvae were inserted, the ends of the tubes were plugged with cotton wool, and the tubes placed in vertical positions.

Attempts were made to use an agar medium to replace the stems, but these were unsuccessful. The best method developed was that in which the stem was replaced by fresh material every twenty-four hours. By this technique, two larvae were kept alive for 22 days, at the end of this period they became mature and ceased feeding.

Although an artificial diet was not developed, it was demonstrated that larvae will survive under these conditions.

Larvae may now be observed while feeding, and many questions concerning behaviour answered. It is felt that if the stem were replaced by some material resembling it in texture and saturated with various nutrients, a truly artificial medium might be developed. As expected, the S-shape has a functional purpose in feeding. The pygidium is braced against the wall of the stem, an opposing thrust is provided by the ventral surface of the abdomen against the opposite wall. The anterior part of the body is curved so the mandibles may be inserted in the plant. Plant tissues are removed in a tearing motion, the head is raised slightly, the tissues are ingested and the mandibles again plunged into the wall.

Two larvae were placed in one tube, both with their heads directed upwards in the stem. The posterior larva, on coming in contact with the anterior, proceeded to feed on the caudal end of its victim. The anterior larva gave some indications of discomfort but made no effort to turn on its attacker. Cannibalism undoubtedly provides a supplementary source of food to the larva, but is not essential as Farstad (32) reports that a normal adult will develop where only one egg was laid in a single stem.

The larva is a voracious feeder for the tubes were often completely filled with frass within twenty-four hours, and in forty-eight hours little remained of the stem but hypoderm. In some tubes even the cotton plugs had been partially ingested and

excreted, apparently unchanged.

TISSUES OF THE STEM INGESTED BY THE LARVA

No attempt has been made to determine the nutrients utilized by the larva. As stated in the introduction, one of the purposes of this project was to determine the tissues ingested by *C. cinctus* larvae. To carry out this aim, examinations were made of infested stems, contents of the larval gut, and tissues in the frass.

Cross-sections of the stem reveal that the larva removes parenchyma and vascular tissues. Parenchyma cells form the largest part of the tissues removed. The larva seldom removes the hypoderm, although Farstad (32) reports that during very dry weather the larva will chew through the hypoderm and into the leaf sheath. One stem showing this type of feeding is illustrated (fig. 32, A), and the normal type of feeding is shown in figures 31, and 32, B. Sterile white heads are produced as a result of this abnormal type of excessively deep feeding. On passing through the node, larvae tunnel through the bundles at the base, and as they ascend they remove only parenchyma (fig. 33). As the larva becomes more mature, it removes parenchyma and vascular tissue to such an extent as to render the stem translucent. The vascular bundles are severed at intervals throughout the internode.

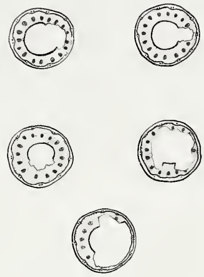

Fig. 31. - Wheat stems infested by C. cinctus. Cross sections of internodes showing typical feeding by the larva.

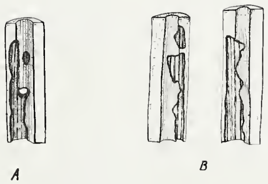

Fig. 32. - Wheat stems infested by C. cinctus. Longitudinal sections of internodes showing feeding. A, larvae cutting through hypoderm; B, typical tunnelling, one stem sectioned in half.

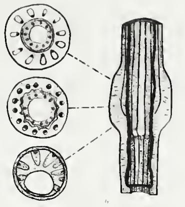

Fig. 33. - Wheat stems infested by C. cinctus. Longitudinal and cross sections showing path of larva through the node.

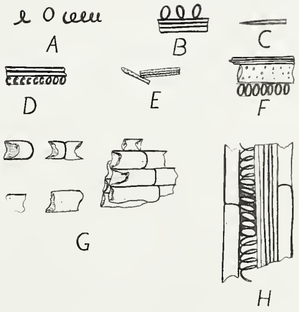

Fig. 34. - Tissues of wheat stem ingested by the larva. A, primary xylem, annular and spiral vessels; B, D, annular vessel and fibres; C, fibre; E, tracheids; F, annular vessel, pitted vessel; fibres; G, parenchyma; H, large fragment of vascular and parenchyma tissue. (All found in the hind-gut).

The gut of the feeding larva is visible through the body wall as a large green tube. In the mesenteron, parenchyma and vascular tissues are found in fragments as large as 0.6 mm. by 0.3 mm. (fig. 34). Twisted and distorted cells, parts of cell walls and mascerated green tissue constitute most of the contents. The hind-gut contains mainly the remnants of cells, mascerated tissue, and some green matter, although this occurs in smaller quantities than in the mid-gut. The larger fragments apparently pass unchanged through the gut. Parenchyma forms the largest part of the food in the intestinal tract.

Tissues in the frass were found to be the same as those in the hind-gut being made up of fragments of cells and mascerated tissue.

From these examinations it is concluded that the larva ingests parenchyma and vascular tissue. Large fragments of plant tissue may pass unchanged through the gut. Apparently the nutrients are obtained from inclusions of cells ingested, among which are green chloroplastids which give the gut its characteristic green colour. Some digestion of the cell walls may take place, although this is not apparent from microscopic examination.

DIGESTIVE ENZYMES

MATERIALS AND METHODS

The insect tissue to be tested was dissected out, fat

body removed as well as possible, and the tissue triturated in glycerol for proteolytic enzymes, in distilled water for lipeolytic and carbohydrate enzymes. In all tests, tissue suspensions were prepared within two hours after larvae were killed. The suspensions were immediately covered with a layer of toluol to prevent bacterial decomposition. The extract was divided into two parts, one part boiled to serve as a control, the unboiled as the test. Substrates were then added, the tubes corked, and placed in a temperature cabinet for incubation.

For pepsin a few drops of hydrochloric acid and a flake of carmine fibrin were added to the suspension. Release of the stain on incubation indicates digestion of the fibrin by pepsin. To test for trypsin five drops of 0.5 per cent sodium carbonate and a flake of congo red fibrin were added to the suspension. The action of trypsin is indicated by release of the stain.

In the test for amylase, a 1 per cent starch solution was added in quantities equal to the suspension. After incubation, it was boiled with Benedict's reagent for reducing sugars, a positive test indicating that the starch had been hydrolysed by amylase. Benedict's reagent was also used in testing for the action of sucrase and cellulase. The substrate of sucrose was made of 10 drops of a 2 per cent solution added to the tissue suspension. For cellulase, quarter-inch squares of filter paper were shredded into fine fragments and added to the tissue suspension.

In testing for maltase and lactase, Barfoed's reagent for the presence of monosaccharides was used after the period of incubation. For maltase, a 1 per cent solution of maltose was added in quantities equal in volume to the suspension. A 1 per cent solution of lactose was added in volume equal to that of the suspension, as the test for lactase.

To test for lipase, 10 drops of just alkaline milk containing phenopthalein was added to the suspension. The action of lipase on milk produces fatty acids, the presence of which are indicated by a disappearance of the pink colour.

The enzymes in these experiments have been named according to the substrate on which they act, although these enzymes may be capable of hydrolysing other substances of related chemical structure. All larvae used in these tests were in the feeding stage.

CARBOHYDRATE ENZYMES

Table I shows the results obtained when complete larvae were used to make up the tissue suspension. Amylase, sucrase, and cellulase were established to be present in the larva.

In Table II, results are recorded when complete alimentary tracts were used to make the tissue extract. Sucrase was established to be present in the gut. Amylase was also found; although negative results were obtained in two tests, it is felt that these results were influenced by experimental conditions,

probably time of incubation and temperature. The presence of cellulase in the gut was not definitely established as the majority of the tests gave negative results. However these results may again be produced by unfavourable experimental conditions. As three tests gave positive results, the presence of a cellulase in the gut is quite probable. The relation between cellulase and micro-organisms will be discussed later.

In Table III, the presence of sucrase in the mesenteron was established. The difficulty encountered in demonstrating the presence of amylase was probably a result of insufficient periods of incubation.

Hind-guts were used for experiments reported in Table IV. Negative results were obtained for the enzymes tested.

Amylase and sucrase were found to be present, cellulase absent, in the labial glands (Table V).

LIPEOLYTIC ENZYMES

The presence of lipase is not established (Table VI).

PROTEOLYTIC ENZYMES

As expected, negative results indicated the absence of pepsin. Difficulty was experienced in the detection of trypsin as the dye was released in both the control and test (Table VII).

CARBOHYDRATE ENZYMES IN THE WHEAT STEM

Tests were carried out using sections of Thatcher wheat stems ground up in distilled water. Starch and sucrose were used as substrates, methods being the same as previously described. It was established that both amylase and sucrase were present (Table VIII).

SUMMARY OF DIGESTIVE ENZYMES

It is established that *C. cinctus* larvae during the feeding stage contain substances which are capable of hydrolysing starch, sucrose, and cellulose. Amylase and sucrase were found in the mid-gut and labial glands, but not in the hind-gut. These enzymes are also present in the wheat stem and may be ingested with the food. The presence of cellulase is indicated in the gut, but has not been definitely established. However, protozoa were found in the gut of some of the overwintering larvae. They occurred in a frequency which was great enough to indicate their presence was not purely adventitious. Some feeding larvae were examined, but protozoa were not observed. However, it is quite possible that these protozoa, which are highly mobile, may be associated with digestion of cellulose. If this is true, then these organisms could quite easily escape during dissection, resulting in a negative test for the hydrolysis of cellulose. This would account for conflicting results obtained in Table II, and uniformly positive results in Table I.

Table I

Tests for carbohydrate enzymes with whole larvae

Date	No. of larvae	Extract (ccs.)	Time incubated (hrs.)	Temperature incubated (^{o}F.)	Substrate									
					Starch		Sucrose		Cellulose		Lactose		Maltose	
					con.*	test	con.	test	con.	test	con.	test	con.	test
1947														
25 Jul.	12	10	2	97	-**	+***								
	15	10	2	97	-	+								
27 Jul.	12	10	1	97	-	+								
28 Jul.	15	10	1	97	-	+								
	10	10	1	97			-	+						
29 Jul.	20	20	24	80					-	+				
31 Jul.	20	20	24	80					-	+				
	10	10	2	97			-	+						
1948														
29 Jul.	20	15	24	70	-	+	-	+	-	+	-	-		
31 Jul.	20	20	24	70	-	+			-	+				
3 Aug.	20	10	48	91					-	+				
9 Aug.	5	2	72	95									-	-

* control
** negative
*** positive

Table II

Tests for carbohydrate enzymes with complete larval gut

Date 1948	No. of guts	Extract (ccs.)	Time incubated (hrs.)	Temperature incubated (^{o}F.)	Substrate							
					Starch		Sucrose		Cellulose		Lactose	
					con.	test	con.	test	con.	test	con.	test
22 Jul.	15	8	10	70	-	-	-	+				
27 Jul.	10	10	24	70			-	+				
29 Jul.	10	10	24	70					-	-	-	-
1 Aug.	15	10	24	70	-	+			-	-		
3 Aug.	15	10	48	91					-	+		
5 Aug.	15	10	48	91					-	+		
	10	5	12	91	-	-	-	+				
9 Aug.	10	5	72	95	-	+			-	-		
10 Aug.	10	10	48	95	-	+			-	-		
12 Aug.	6	4	48	95	-	+			-	+		
	* 5	5	48	95	-	+			-	-		
	* 10	10	72	95	-	+			-	-		
13 Aug.	12	5	24	95	-	+			-	-		
31 Jul.	** 15	9	24	70	-	+			-	-		
6 Aug.	** 12	6	48	95	-	+			-	-		

* Guts opened and contents washed out

** Complete larval guts and salivary glands

Table III

Tests for carbohydrate enzymes with mid-guts

Date 1948	No. of mid-guts used	Extract (ccs.)	Time incubated (hrs.)	Temperature incubated (^{o}F.)	Substrate					
					Starch		Sucrose		Cellulose	
					con.	test	con.	test	con.	test
23 Jul.	10	10	12	70	-	-	-	+		
28 Jul.	10	5	24	91	-	-	-	+		
5 Aug.	15	10	48	91	-	+	-	+	-	-
26 Jul.	* 10	10	24	70					-	-

* Mid-guts and hind-guts

Table IV

Tests for carbohydrate enzymes with hind-guts

Date 1948	No. of hind-guts used	Extract (ccs.)	Time incubated (hrs.)	Temperature incubated (°F.)	Substrate					
					Starch		Sucrose		Cellulose	
					con.	test	con.	test	con.	test
26 July	10	10	24	70					-	-
28 July	10	5	24	91	-	-	-	-		
5 Aug.	15	9	48	91	-	-	-	-	-	-

Table V

Tests for carbohydrate enzymes with labial glands

Date 1948	No. of labial glands	Extract (ccs.)	Time incubated (hrs.)	Temper-ature incubated (^{o}F.)	Substrate					
					Starch		Sucrose		Cellulose	
					con.	test	con.	test	con.	test
28 July	10	5	24	91	-	+	-	+		
5 Aug.	15	9	48	91	-	+	-	+	-	-
13 Aug.	12	5	24	95	-	+			-	-

Table VI

Tests for lipeolytic enzymes

Date	Number	Part used	Extract (ccs.)	Time incubated (hrs.)	Temper-ature incubated (°F.)	Substrate Milk	
						control	test
1947							
29 July	15	Larval guts	10	1	97	-	-
1948							
27 July	10	Complete larval guts	10	24	70	-	-
31 July	10	Gut and labial glands	10	24	70	-	+
1 Aug.	15	Complete guts	10	24	70	+	+
3 Aug.	20	Complete larvae	20	48	91	-	-

Table VII

Tests for proteolytic enzymes

						Substrate			
						Carmine fibrin and HCl.		Congo red fibrin and Na_2CO_3	
Date	Number	Part used	Extract (ccs.)	Time incubated (hrs.)	Temperature incubated ($^{\circ}F.$)	(pepsin) con.	test	(trypsin) con.	test
1947									
27 July	10	Larval guts	5	1	97			-	-
28 July	15	Larval guts	10	1	97			+	+
1948									
27 July	15	Larval guts	10	24	70	-	-	+	+

Table VIII

Tests for carbohydrate enzymes in the wheat stem
(Thatcher variety)

Substrate	Control	Test
1% starch solution	-	+
2% sucrose solution	-	+

SUMMARY AND CONCLUSIONS

1. The morphology and histology of larval organs which carry out ingestion, digestion, and excretion, are described.

2. A method is described whereby the feeding stages of *C. cinctus* larvae may be observed while feeding, moulting, and perhaps producing hibernacula.

3. It was determined that larvae of *C. cinctus* secure from parenchyma and vascular tissues of the wheat stem, all nutrients required for growth, development, and reproduction.

4. Substances capable of hydrolysing starch and sucrose were established to be present in the mesenteron and labial glands of the feeding stage of *C. cinctus* larvae. Cellulose was found to be hydrolysed in the larva, probably in the alimentary canal, and the hydrolysis may be associated with protozoa found in the larva.

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